Low Concentration Digest of

D5581-D5675

This protocol assumes that you have read and understand the manufacturer's instructions attached below. Please read the full manufacturer's instructions before using this abbreviated protocol.

Digest recipe 50µL rxn - use digest sheet in PCR worksheet, double check math: (https://docs.google.com/spreadsheets/d/1LGt2WziwmGoJMluBcwmKfhZrGjZOlJLkVHf59-5cOV4/edit?usp=sharing) or the R code below

Fill in the number of samples and the code will do the rest.

```
plate <- "D5581-D5675"
num_samples <- 93
```

Setup						
number_samples	error	reaction_size	enzyme1_conc	enzyme2_conc	mstr_mx_ppt_vol	unts_ech_enzyme
93	102.3	50	1e+05	10000	20	20

Per Sample				
uL_sample	ul_enzyme1	ul_enzyme2	ul_buffer	ul_pH2O
30	0.1	1	5	13.9

Master Mix				
ul_enzyme1	ul_enzyme2	ul_pH2O	ul_buffer	total_volume
10.23	102.3	1421.97	511.5	2046

Divide	
wells_8	wells_16
255.75	127.875

- Make the master mix recipe in 15mL falcon tube by following the amounts on the spreadsheet above
- split the master mix into 8 wells
- use a multichannel pipet to pipet 20µL of master mix into each sample well.
- Do not add the master mix to all of the wells and then add the sample. Because you are working with active enzymes, they should be the last things added to the master mix and the last step of plate preparation.
- Incubate PstI and MluCI at 37 ˚ for one to four hours - No benefit from incubating MluCI longer than one hour, PstI is active 2-4 hours, don't need to heat kill digests.
- Clean and quantify the digests.